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## **Introduction**

The purpose of the proposed research is to establish the cDNA microarray technology in the study of mouse mammary tumor development induced by Wnt1 proto-oncogene. Gene expression profiles will be established for mammary tumors induced by Wnt1 and other oncogenes whose expression is known to induce mammary tumors as well (i.e. c-myc, ras, neu, and erbB2), and by Wnt1 in cooperation with either Fgf-3 transgene or p53 null mutation. Comparison of these expression profiles will identify common genes that are altered in all samples as well as differentially expressed genes in a particular tumor. Such analysis may provide insight into mechanisms underlying tumorigenesis and molecular markers for diagnosis and prognosis of mammary tumors.

We also propose to use the cDNA microarray to isolate and characterize responsive genes downstream of the Wnt signal transduction pathway. cDNA microarray will be used to monitor the expression of about ten thousand genes and their expression patterns compared before and after the acute activation of Wnt signaling in mammary epithelial cells. Similar experiments will also be carried out with the human embryonic kidney epithelial 293 cells using the human cDNA microarray which is currently available to us. The identification and further characterization of the potential target genes may help us understand how Wnt proteins, and presumably other proteins including beta-catenin in the Wnt signaling pathway, transduce signals into the nucleus and invoke gene responses. These research may also provide new targets for intervention and novel strategies for treatment of mammary tumors.

## Annual Summary

### **Technical Objective 1. Construct cDNA Microarray To Be Used in Studying Gene Expression In Wnt-1 Induced Mouse Mammary Tumors**

#### *1.i Construct the cDNA Microarray*

Status: Completed.

I have completed the Technical Objective 1.i as outlined in the Statement of Work in collaboration with Dr. Bill Pavan's lab at the National Human Genome Research Institute (NHGRI). The current prints of the mouse cDNA microarray chips contain about 5,000 mouse unigene cDNA clones. PCR fragments of those unigene cDNA clones were amplified in a 96 well format with the M13 forward and reverse primers. Amplified PCR fragments were then printed on the pre-treated glass slides using a custom-made microarray printer located in Dr. Jeff Trent's laboratory at NHGRI. I am also preparing to print another batch of the mouse microarray prints that will contain about 8,000 mouse unigene cDNA clones that have been sequence verified and currently being developed in Dr. Bill Pavan's lab.

#### *1.ii Test Microarray And Optimize Assay Conditions*

Status: In progress

I have dissected the normal and tumor mammary gland tissue samples from the Wnt-1 transgenic mice. Total RNA was prepared from these samples using Trizol reagent (Gibco BRL). RNAs were used as templates for the fluorescent probe synthesis. Fluorescence-tagged probes were subsequently hybridized to the mouse cDNA microarray chips and the hybridization results obtained after the chips were scanned for both the cy3 and cy5 channels. Currently, I am in the process of optimizing the labeling and hybridization conditions to achieve reproducible results for the microarray experiments.

#### *1.iii Test-Drive Microarray In The Study Of Differential Gene Expression In Mammary Tumor Cells.*

Status: In progress.

As mentioned above, I have dissected both the normal mammary gland tissue and the Wnt-1 transgenic mammary gland tumor samples from the Wnt-1 transgenic mice. I am currently optimizing the labeling and hybridization conditions and trying to achieve reproducible hybridization results.

## **Technical Objective 2 Analyze Differential Gene Expression Patterns In Various Mammary Tumor Samples.**

Status: To be performed.

Once the labeling and hybridization conditions are optimized for the mouse tissue samples, the experiments outlined in this objective will be carried out immediately.

## **Technical Objective 3 Identify And Characterize Potential Wnt-1 Responsive Genes.**

### *3.i Establish Paracrine System To Activate Wnt-1 Signaling In C57MG Cells.*

Status: Completed.

I have also moved objective 3.i ahead in order to establish a tissue culture system in which the acute Wnt-1 signaling can be achieved. QT6 cells expressing either Wnt-1 proteins have been obtained and their expression was confirmed by Western blot analysis. Activities of Wnt-1 proteins produced by the QT6 cells were tested in paracrine assays by co-culturing QT6 cells expressing Wnt-1 proteins with the C57MG mouse mammary gland epithelial cells (responder cells). Active Wnt-1 proteins should be able to transform responder C57MG cells as reported before. Indeed, this was the observed results and C57MG cells were readily transformed by QT6 cells expressing Wnt-1 proteins but not by the QT6 parental cells. Although Wnt-1 proteins produced by the QT6 cells clearly activated Wnt-1 signaling pathway and transformed C57MG cells in those co-culture experiments, I could not detect any significant beta-catenin stabilization in this assay. This indicates that Wnt-1 activity was most likely restricted to the C57MG cells that were immediately adjacent to the Wnt-1 producing QT6 cells. Repeated efforts to optimize co-culturing conditions such as to enable more C57MG cells to receive Wnt-1 signaling have thus far been unsuccessful. Therefore, I have decided to abandon the proposed paracrine co-culture strategy to initiate acute Wnt-1 signaling in C57MG cells.

## **Additional Technical Objectives Not Included In the Statement of Works.**

### *1. An Alternate Strategy To Initiate Wnt-1 Signaling In C57MG Cells.*

Status: In progress.

To circumvent the encountered difficulties in initiating Wnt-1 signaling in C57MG cells by paracrine co-culture, I plan to employ the TV-A system to express Wnt-1 protein and initiate Wnt-1 signaling in C57MG cells. TV-A protein encodes a receptor for the avian leukosis virus and is expressed only in avian but not mammalian cells. Therefore, expression in mammalian cells allows high-efficiency, specific infection by virus carrying genes of interest. Dr. Mario Chamorro in the lab has successfully generated C57MG cells expressing the TV-A receptors. These cells were shown capable of being infected by viruses that carried the alkaline phosphatase marker gene with nearly 100% efficiency. I plan to collaborate with Dr. Mario Chamorro and deliver Wnt-1 gene (or other genes that are involved in the Wnt signaling pathway such as beta-catenin and lef-1

genes) into these cells to initiate acute Wnt-1 signaling. RNA prepared from the viral infected C57MG cells will then be compared to the mock infected C57MG cells in the cDNA microarray experiments. Potential responsive genes will be verified by Northern blot analysis, and their function tested in Wnt-1 related biological and biochemical assays.

## *2. Generation Of Human 293 Cells That Inducibly Express Wnt-1, Beta-Catenin, And Lef-1 Proteins*

Status: Completed.

While preparing and constructing mouse cDNA microarray, I took advantage of the existing sequence-verified human unigene cDNA microarray to identify Wnt-1 downstream target genes in human kidney embryonic epithelial 293 cells. Ecdyson inducible system was employed to generate 293 cells that inducibly express either Wnt-1, beta-catenin, or Lef-1 proteins. Several independent clones have been obtained which express Wnt-1, beta-catenin, or Lef-1 proteins after the addition into the media of inducer ponasterone. Northern blot analysis showed that expression of Wnt-1 protein was induced as early as 4 hours post-induction. Induced expression of Wnt-1 protein stabilized both the cytoplasmic and nuclear beta-catenin suggesting that induced expression of Wnt-1 protein can initiate Wnt-1 signaling in 293 cells. Consistent with this notion, induced expression of Wnt-1 protein also activated the Lef/TCF reporter gene by nearly ten folds indicating that induced expression of Wnt-1 proteins not only stabilized beta-catenin but also can activate downstream gene expression.

## *3. Identification Of Downstream Target Genes From The Wnt-1 Signaling Pathway Use Human Unigene cDNA Microarray*

Status: In progress.

Total RNAs have been prepared from 293 cells before and after the induced expression of Wnt-1 or beta-catenin proteins. cDNA microarrays containing the human unigene cDNA clones were then utilized to identify genes that are either up-regulated or down-regulated by the induced expression of Wnt-1 or beta-catenin in 293 cells. Currently, we are verifying several genes that showed consistent up-regulation by the induced expression of either Wnt-1 or beta-catenin proteins, although two of those genes have thus far failed to be verified by Northern blot analysis. I am currently preparing to repeat these experiments with a different print of microarray chips since some of the false positives might be print dependent.



## **Appendix**

### **1. Key research accomplishments**

- Completed printing the mouse unigene cDNA microarray chips containing 5,000 genes.
- Established inducible cell lines to express Wnt-1, beta-catenin, and Lef-1 proteins.

### **2. Reportable outcomes**

None.